U.S. NATIONAL SCIENCE FOUNDATION
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NSF 24-084

Dear Colleague Letter: Posttranscriptional and Posttranslational Modification (PPM)

May 02, 2024

Dear Colleagues:

Presidential Executive Order (EO) 14081 on "Advancing Biotechnology and Biomanufacturing Innovation for a Sustainable, Safe and Secure American Bioeconomy" envisions a vital role for biotechnology in harnessing the power of biology to benefit society. From mitigating climate change to ensuring food security, improving human health, and creating resilient supply chains, understanding the mechanisms in cells and organisms that shape how genotype becomes phenotype, and how cellular information is transformed into organismal fitness and performance is central to achieving breakthroughs in biotechnology and growing the bioeconomy. The central dogma of molecular biology has expanded well beyond the flow of information from DNA-to-RNA-to-protein into a system of complex interactions and feedback loops among all these macromolecules and, importantly, their many isoforms and chemically modified variants. Post-transcriptional RNA modifications (PRM) and post-translational protein modifications (PTM) are essential mechanisms used by cells to diversify and regulate the structure and function of RNA and proteins, and to coordinate regulatory and signaling networks. Beyond post-transcriptional and post-translational processing, such as RNA splicing or signal peptide cleavage, the many, possible combinations of covalent PRM and PTM modifications provide a critical mechanism for defining, as well as modulating, cellular and organismal states and functions, and numerous developmental and physiological disorders have been linked to defects in PRM and PTM pathways. **The challenge now is to understand how these modifications interact with one another and create multi-layered, dynamic, regulatory networks within cells, that govern organismal phenotype**.

The chemical properties of RNA enable a large diversity of structures and roles in biological systems, from coding information to scaffolding to catalysis. This broad palette is further enhanced through site-specific addition of over 170 currently known PRMs. These covalent modifications are found in all major RNA classes (tRNA, mRNA and rRNA) across all three domains of life (archaea, bacteria and eukaryotes), as well as in small RNAs (e.g., miRNA

and snRNA) and long noncoding RNAs. In addition to questions about modification mechanisms and impacts on RNA structure and function, how multiple modifications influence each other on the same or different RNAs, why the same modification can have different effects on different classes of RNA (e.g., miRNA vs. tRNA), how these PRM affect RNA interactions with proteins and DNA, and ultimately how they define cellular and organismal functions all remain largely unexplored.

Likewise, PTMs extend the chemical repertoire of the 20 standard amino acids by over 400 known covalent modifications. PTMs influence all aspects of protein structure and function and, thus, cellular and organismal biology. While modifications of a few protein classes, such as kinases and histones, have been studied extensively, much less is understood about the PTMs of many other proteins. Moreover, how multiple modifications on individual proteins interact to impact assembly, structure and function of complexes and proteomes is also largely unexplored. Finally, how modified RNAs and proteins work together also remains fertile ground for investigation.

With this Dear Colleague Letter (DCL), NSF invites proposals that address understanding how multiple modifications of single or multiple, functionally-related RNAs and/or proteins influence the properties, interactions, and/or regulation of these macromolecules, and ultimately their role in cellular and organismal phenotype.

Examples of areas of interest include, but are not limited to:

- Signals, processes, and rules governing the identity, position, number, and structure/function consequences of modifications on RNA and proteins;
- Whether integrated, systems-level effects of multiple modifications on RNA and proteins are additive, cooperative or competitive, and why and how this impacts phenotype, in any given case;
- Roles of multiple PRM and/or PTM on cell and/or organismal sensing, fate and function in a dynamic environment;
- Design and engineering of synthetic biology systems to study multiple RNA and protein modifications; programmable systems that leverage RNA and protein modifications to control cell or organismal fate and function.

While strategies developed to characterize single RNA and protein modifications can be applied to populations of molecules, the characterization of multiple modifications on individual RNA or proteins in a quantitative way is only just beginning. Advancing frontiers in this field will add new strategies, methodologies, and computational approaches to those currently available (e.g., mass spectrometry, stable isotope labeling, peptide sequencing, etc.) to detect, analyze, manipulate, model, and engineer the PRMs, PTMs and biological systems to be studied. Proposals that pursue novel methods or technologies are welcome, but should be motivated by a specific research question or hypothesis that is not accessible

by currently available tools. Higher priority will be given to proposals that tackle complexity on a systems scale and develop or apply innovative, interdisciplinary approaches. For this funding opportunity, projects focused on relatively well-studied, single modifications, for example, the role of a specific modification on a histone tail, will be given lower priority. Successful proposals will present a compelling vision of how the proposed work will yield fundamental discoveries that can benefit society

PROPOSAL SUBMISSION AND REVIEW

Proposals must follow the guidelines and any solicitation specific criteria, if applicable, of the relevant NSF program most closely related to the research. The title of a proposal submitted in response to this DCL must begin with "PPM" after any solicitation-specific title requirements, if applicable. The participating programs are:

- Division of Integrated Organismal Systems (IOS) Core Programs
- Division of Molecular and Cellular Biosciences (MCB) Core Programs
- Division of Biological Infrastructure (DBI), Infrastructure Innovation for Biological Research (Innovation)
- Division of Chemical, Bioengineering, Environmental and Transport Systems (ENG/CBET), Cellular and Biochemical Engineering (CBE)
- Division of Chemistry (CHE), Chemistry of Life Processes (CLP)

Proposals submitted in response to this DCL will be reviewed alongside other proposals submitted to the relevant program, and will be reviewed in accordance with NSF's review criteria for Intellectual Merit and Broader Impacts and any solicitation specific review criteria, if applicable. Investigators are strongly encouraged to contact a program director of the relevant program before submitting a proposal. Divisional representatives of participating programs are:

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Sincerely,

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